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1 **Title:**

2 New perspectives on nutritional interventions to augment lipid utilisation during exercise

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18 **Running title:** Nutritional modulation of exercise metabolism

19

20 **Key words:** Glycaemic index, L-carnitine, Calcium, Fish oil, Caffeine

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22

23 **Abstract:**

24 The enhancement of fat oxidation during exercise is an aim for both recreational exercising
25 individuals and endurance athletes. Nutritional status may explain a large part of the variation in
26 maximal rates of fat oxidation during exercise. This review reveals novel insights into nutritional
27 manipulation of substrate selection during exercise, explaining putative mechanisms of action and
28 evaluating the current evidence. Lowering the glycaemic index of the pre-exercise meal can
29 enhance lipid utilisation by up to 100% through reduced insulin concentrations, although its
30 application may be restricted to specific training sessions rather than competition. Chronic effects of
31 dietary glycaemic index are less clear and warrant future study before firm recommendations can be
32 made. A flurry of recent advances has overthrown the conventional view of L-carnitine
33 supplementation, with skeletal muscle uptake possible under certain dietary conditions and
34 providing a strategy to influence energy metabolism in an exercise intensity-dependent manner. Use
35 of non-carbohydrate nutrients to stimulate muscle L-carnitine uptake may prove more beneficial for
36 optimising lipid utilisation but requires more research. Studies investigating fish oil
37 supplementation on fat oxidation during exercise are conflicting. In spite of some strong putative
38 mechanisms, the only crossover trial showed no significant effect on lipid use during exercise.
39 Calcium may increase NEFA availability although it is not clear whether these effects occur
40 Calcium and caffeine can increase NEFA availability under certain circumstances which could
41 theoretically enhance fat oxidation, yet strong experimental evidence for this effect during exercise
42 is lacking. Co-administration of nutrients to maximise their effectiveness needs further
43 investigation.

44

Maximum rates of fat oxidation during exercise vary from 0.18 to 1.01 g·min⁻¹, more than a 5-fold difference⁽¹⁾. Training status, lean body mass, estimated physical activity level, gender and fat mass account for only 34% of the variance. Hence, along with heritability, nutrition has the potential to largely influence fat oxidation. Indeed, acute pre-exercise nutrition may in fact override some of the training-induced adaptations in gene transcription related to substrate metabolism⁽²⁾. The limitations to fat oxidation vary depending on the intensity of exercise and have been reviewed extensively elsewhere^(3; 4). Maximal fat oxidation rates occur during exercise between 45 and 65% VO_{2max} and are drastically reduced to minimal rates above 85% VO_{2max}. Reduced plasma NEFA availability may explain part, but not all⁽⁵⁾, of the decrease. Fatty acid transport across muscle and mitochondrial membranes are thought to be important rate limiting steps. These restrictive processes are discussed in more detail later in context of the nutritional manipulation involved. The rate of fatty acid flux may modulate the improvements in insulin sensitivity and glucose tolerance seen with an exercise training programme^(6; 7). Thus, enhanced fat oxidation could be seen as a goal for both athletes and the general population for enhancing adaptation to exercise which can potentially improve performance for the former, and health for the latter.

The broad-ranging relevance of enhanced fat oxidation, combined with the complex integration of biological systems and limiting step behind its regulation⁽⁸⁾, makes this an interesting and important topic for research and has led to the construction of some previous reviews on dietary strategies^(9; 3). Yet, since the publication of these articles, some exciting advances have occurred in this area. The aim of this review is to provide new insights into the manipulation of lipid oxidation via nutrition in the peri-exercise period and to supply potential mechanisms by which these effects occur (Figure 1). Further intentions include the emphasis of crucial gaps in knowledge such as the optimal timing and potential co-ingestion of nutrients. If these points are addressed, the external validity of nutritional manipulations can be extended to athletes and recreational exercisers.

Glycaemic index

Consumption of carbohydrate containing foods results in an increase in blood glucose concentration, in turn stimulating insulin release by the β -cells of the pancreas. The glycaemic index is a method of classifying carbohydrates according to their blood glucose response⁽¹⁰⁾. Consumption of low glycaemic index (LGI) carbohydrates results in a smaller glycaemic response than an equal quantity of high glycaemic index (HGI) carbohydrates. This can be due to a variety of factors, including the physical texture⁽¹¹⁾ and chemical structure of the food⁽¹²⁾, the presence of fibre⁽¹³⁾ and organic acids⁽¹⁴⁾. Insulin is known to inhibit lipolysis and thus reduce NEFA

availability⁽¹⁵⁾. This led to the hypothesis that LGI foods can attenuate the suppression of fat oxidation compared to HGI foods.

A review of studies where lipid oxidation was determined under sedentary conditions by Diaz *et al.*⁽¹⁶⁾ concluded that metabolic differences between high and low GI foods were too small to influence fat metabolism. Although outside of the scope of this review of exercise metabolism, a number of useful points can be taken which may explain why no difference was detected with these studies. Of the acute studies reviewed, some used either fairly low absolute (50 g)⁽¹⁷⁾ or high relative (5 g/kg body mass)⁽¹⁸⁾ amounts of carbohydrate. While others used obese subjects⁽¹⁹⁾ who, although displayed normal glucose tolerance, may still have exhibited insulin resistance and metabolic inflexibility⁽²⁰⁾. Therefore, shifts in substrate metabolism from postabsorptive to postprandial states could have been blunted. In others, glucose responses were not evaluated⁽²¹⁾ and hence the results cannot be attributed to GI with certainty. Differences in resting metabolism have been observed with LGI mixed meals^(22; 23). Yet, the increased metabolic flux seen during exercise may accentuate any differences, thus illuminating a change in fat utilisation not apparent at rest.

Early exercise studies showed promising results for augmented fat oxidation, but employed isolated, carbohydrate rich foods (such as lentils only vs potato only)^(24; 25), which, are unlikely to be consumed by the general population in isolation due to low palatability. Therefore, a series of subsequent studies used high carbohydrate mixed-meals containing carbohydrate, protein and fat (contributing 76%, 12% and 12% to total energy content, respectively) from typical breakfast foods such as cereal, fruit and bread^(26; 23) which is pertinent for real-world application. These mixed-meal design studies have demonstrated up to two-fold increases in the amount of whole body fat oxidation during treadmill running⁽²⁶⁾. This occurred during exercise intensities ranging from 50 – 70% $\text{VO}_{2\text{max}}$ ^(21; 24) in both males⁽²⁵⁾ and females^(21; 24), differing in activity levels^(21; 24) and differences even occur during the first 15 min of exercise⁽²⁴⁾. Furthermore, similar findings are seen with carbohydrate intakes ranging from 1 to 2.5 g/kg body mass^(27; 23) and when the pre-exercise meal is ingested from 30 min⁽²⁸⁾, up to 12 h before exercise⁽²⁹⁾. The intakes of carbohydrate used in the exercise studies (1-2.5 g/kg body mass) are within the guidelines for pre-exercise carbohydrate consumption which strengthens the relevance of these studies for everyday use prior to exercise-training.

Mechanisms explaining the increase in fat oxidation during exercise with LGI meals probably include greater availability of NEFAs, and reduced pre-exercise muscle glycogen concentrations

108 compared to HGI meals⁽²⁷⁾. This leads to greater muscle glycogen utilisation and therefore less of a
109 reliance on fat oxidation via reduced AMP-activated protein kinase (AMPK)⁽³⁰⁾.

110 It is appealing to consider that meal timing could be crucial to the effectiveness of GI modulation of
111 substrate metabolism. A low GI meal is known to produce a second-meal phenomena whereby, the
112 glycaemic response to a standard meal is lower following previous consumption of a LGI,
113 compared to a HGI meal. Stevenson *et al.*^(31; 32) examined whether this effect can result in a shift in
114 lipid utilisation during exercise. Participants were given a LGI or HGI evening meal, followed by a
115 standard HGI breakfast the morning after. In spite of a lower glycaemic response to breakfast, no
116 differences in NEFA availability or fat oxidation were observed during exercise. Reasons for this
117 discrepancy are unclear, although there are a number of possible explanations. It could be
118 hypothesised from these studies that LGI foods need to be consumed within the 3 h prior to exercise
119 which would optimise the difference in muscle glycogen and therefore influence substrate selection.
120 On the other hand a LGI diet during recovery from a glycogen-reduction run, led to increased fat
121 usage during fasted exercise the following day⁽²⁹⁾.

122 A few explanations provide a more convincing argument;

- 123 1) Breakfast is the most important time for consuming LGI foods due to being the first meal
124 consumed after the overnight fast. Exposure to NEFAs during fasting can induce insulin
125 resistance⁽³³⁾. Therefore, changes in the insulin response could be enhanced and may provide
126 an opportunity to exploit the difference between the GI of meals along with the allied
127 metabolic consequences. Evidence supporting this is provided by the attenuated disparity in
128 glycaemic response to LGI and HGI meals when consumed at lunch as opposed to
129 breakfast⁽³⁴⁾.
- 130 2) The greater fibre content of the LGI foods may lead to an increase in SCFAs via increased
131 gut fermentation⁽³⁵⁾. Indeed, this is probably a major contributor to the second-meal
132 phenomenon. In an animal model, the SCFA butyrate has been shown to increase lipid
133 utilisation⁽³⁶⁾, and may therefore be an integral aspect in the modulation of substrate
134 utilisation from LGI vs HGI meals. As the glycaemic response to the standard HGI breakfast
135 (which contained equal amounts of fibre) was different⁽³²⁾. This implies that it may not be
136 the glycaemic response *per se*, but the fibre content inherent of many LGI foods.
- 137 3) Finally, whole-grain foods contain a variety of bioactive compounds and micronutrients⁽³⁷⁾
138 which should not be disregarded in terms of their potential to influence metabolism.

139 Interestingly, the fructose load of the LGI breakfasts used in some of these studies was 25 g for a 70
140 kg participant⁽³⁸⁾. Although LGI, fructose inhibits fat oxidation during and after exercise to a greater
141 extent than glucose^(39; 40). This illustrates the powerful influence of GI combined with exercise in
142 these studies^(38; 26), as substrate metabolism was greatly affected, in spite of the large fructose load
143 of the LGI breakfast. In fact, fructose was probably the reason for the greater postprandial blood
144 lactate concentrations⁽⁴¹⁾, which, it should be noted, also inhibits lipolysis⁽⁴²⁾ via the G protein-
145 coupled receptor GPR81^(43; 44). This influence of fructose may also provide an explanation for why
146 some of the resting studies showed no effect of GI on fat metabolism⁽¹⁹⁾. High fructose consumption
147 has been linked to de novo lipogenesis, TAG accumulation and insulin resistance⁽⁴⁵⁾. Therefore, the
148 use of fructose to reduce the GI of a meal may be counterproductive to lipid metabolism not only
149 acutely, but also in the long-term.

150 A few recent studies have found results which, at a glance, conflict with the majority of previous
151 findings. Moore *et al.*⁽⁴⁶⁾ remarkably displayed increased fat oxidation and NEFA concentrations
152 during exercise following a HGI meal compared to a LGI meal. Explanations for this are not easily
153 forthcoming, although, the LGI meal contained a greater quantity of milk. Milk proteins are
154 particularly insulinotropic⁽⁴⁷⁾, which may have caused the greater suppression of NEFAs and
155 therefore fat oxidation. However, this is merely speculation, as insulin concentrations were not
156 measured. Furthermore, the validity of indirect calorimetry to estimate substrate utilisation is
157 dependent upon exercise being “steady-state”. As the exercise in the study by Moore *et al.* was a
158 time trial, power output is likely to fluctuate and hence invalidate the assumptions of indirect
159 calorimetry⁽⁴⁸⁾. This latter explanation may also partially clarify why Little *et al.*⁽⁴⁹⁾ found no
160 difference between HGI and LGI pre-exercise meals on substrate metabolism during high-intensity,
161 intermittent running estimated by indirect calorimetry. Moreover, muscle glycogen was
162 approximately 20% lower in the HGI compared to the LGI trial after 75 min of the protocol (as
163 estimated from the figure in Little *et al.* 2010). Albeit not statistically significant, this is a
164 considerable difference. As it was reported that muscle glycogen was similar pre-exercise (values
165 not given), it could be assumed that the LGI meal did in fact reduce muscle glycogen utilisation,
166 and hence increase lipid oxidation that was not detected by indirect calorimetry. Bennard & Doucet
167 also reported no difference in lipid utilisation between HGI and LGI pre-exercise meals⁽⁵⁰⁾. Yet, the
168 blood glucose response to the “LGI” meal prior to exercise tended to be *greater* than that of the
169 “HGI” meal. Thus, by definition, the LGI meal was not lower in glycaemic index than the HGI
170 meal. It appears that the glycaemic index of the pre-exercise meal can significantly influence
171 substrate selection during exercise, with LGI meals producing a lower insulin-induced suppression

172 of lipolysis and NEFA availability, along with reduced muscle glycogen content compared with
173 HGI pre-exercise meals.

174 When a LGI breakfast and lunch are administered, no difference in fat oxidation occurs during
175 exercise after lunch, compared to HGI breakfast and lunch⁽³⁸⁾. The authors suggested that the
176 intensity of exercise (70% $\text{VO}_{2\text{max}}$) may have been too high for a difference in fat oxidation to
177 occur. Although another study has demonstrated a difference in fat oxidation during similar
178 intensity exercise (71% $\text{VO}_{2\text{max}}$) after a single LGI vs. HGI meal⁽²⁷⁾, the participants differed in
179 training status ($\text{VO}_{2\text{max}}$: 55.1 vs 64.6 ml/kg/min). Training status is known to enhance fat utilisation
180 at the same relative intensity⁽³⁾, which may explain this incongruity. Increased fat oxidation in the
181 postprandial period following lunch makes it tempting to speculate that a lower intensity of exercise
182 for this population would have led to differences in substrate selection to become apparent.

183 In contrast to acute feedings, dietary glycaemic index and fat oxidation during exercise has, to date,
184 received relatively little research interest. To our knowledge, two studies have investigated the
185 effects of dietary GI on whole body fat oxidation during exercise and both found no effect of diet^{(51;}
186 ⁵²⁾. However, the relatively short time period (3 & 5 d), combined with the high carbohydrate loads
187 (10 and 7 g/kg body mass/d) may have restricted the ability to identify differences between diets.
188 The only study to date, in which LGI and HGI diets were provided, alongside an aerobic exercise
189 programme found a greater increase in $\text{VO}_{2\text{max}}$ and greater reduction in systolic blood pressure with
190 a 7-d LGI compared to a HGI diet⁽⁵³⁾. Although no difference in postprandial resting fat oxidation
191 was reported. When the study was extended to 12 weeks, the differences in $\text{VO}_{2\text{max}}$ and blood
192 pressure were abolished, yet the LGI diet contributed to the improved insulin sensitivity with
193 exercise training⁽⁵⁴⁾. It would be interesting to explore this further and investigate whether GI can
194 influence adaptations to an exercise programme. A plausible rationale exists; increasing fat
195 oxidation during an exercise programme, via reduced carbohydrate availability and/or increased
196 NEFA availability, could enhance the increase in mitochondrial enzyme activity^(55; 56; 57). If the
197 glycaemic index of the pre-exercise meal can be used to alter substrate availability, then this may be
198 a novel method of maximising the benefits of an exercise programme for the general public.

199

200 It is apparent that pre-exercise LGI meals do lead to a greater rate of fat oxidation during sub-
201 maximal, continuous exercise. Although questions remain unanswered: is the effect only observed
202 with the first meal after an overnight fast, or the last meal before exercise? The acute applications of
203 glycaemic index in terms of sports nutrition are probably limited as, in most sports, there are no

204 limitations on nutritional intake during exercise. Carbohydrate consumption during exercise
205 nullifies the influence of a pre-exercise meal GI on substrate metabolism^(58; 59; 60; 61), thus, the
206 application may be limited to endurance events where optimal nutrient intake during competition is
207 prohibited or impractical (eg. the swim leg of an Ironman triathlon, long-distance open-water
208 swimming). Notwithstanding this, exercise training on a LGI diet may still be pertinent for
209 enhancing endurance adaptations and for improving health of the general public and chronic trials
210 are needed to confirm the effect of consuming a LGI diet in combination with exercise.

211

212 **L-carnitine**

213 The amino acid carnitine exists in two isoforms. While D-carnitine is thought to be biologically
214 inactive⁽⁶²⁾, L-carnitine plays an important role in enabling the transport of long-chain acyl-CoA
215 across the otherwise impermeable inner mitochondrial membrane. Carnitine palmitoyltransferase 1
216 (CPT1) catalyses the esterification of free carnitine, with long-chain acyl-CoA⁽⁶³⁾. The long-chain
217 acylcarnitine is subsequently transported across the mitochondrial membrane, in concurrent
218 substitution with free carnitine from inside the mitochondrial matrix. Once inside the
219 mitochondrion, carnitine palmitoyltransferase 2 (CPT2) catalyses the transesterification of long-
220 chain acylcarnitine back to free carnitine and long-chain acyl-CoA⁽⁶⁴⁾, which is then able to enter
221 the β -oxidation pathway. A thorough review of the role of L-carnitine in fatty-acid transport is
222 provided by Stephens *et al.* ⁽⁶⁵⁾.

223 Muscle free carnitine content is reduced during high-intensity exercise and when muscle glycogen
224 content is elevated. This has led to suggestions that free carnitine content may limit the rate of fat
225 oxidation via reduced CPT1-mediated mitochondrial membrane transport. This has been
226 demonstrated in vitro, where elevating the free carnitine pool raises long-chain fatty acid oxidation
227 by mitochondria.

228 In vivo studies have had less success. A previous review concluded that L-carnitine
229 supplementation is not effective⁽⁹⁾. This is due to the fact that oral L-carnitine supplementation *per*
230 *se* is unable to increase intramuscular carnitine content, partly due to the high concentration
231 gradient from muscle ($\sim 3.5 \text{ mmol} \cdot \text{L}^{-1}$) to plasma ($50 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$). Indeed, even when L-carnitine is
232 administered intravenously, muscle carnitine content is unaffected⁽⁶⁶⁾ and thus it is no surprise when
233 differences in lipid utilisation are absent.

234 Intriguingly, recent studies have demonstrated that hyperinsulinaemia, in the presence of
235 hypercarnitinaemia can augment muscle carnitine content by ~14%, probably via an increase in
236 NA⁺/K⁺ pump activity^(66; 67). In addition, the increase in muscle carnitine content resulted in a 30%
237 increase in muscle glycogen content, thus resulting in the exciting proposition that lipid oxidation
238 was indeed enhanced, leading to reduced carbohydrate utilisation⁽⁶⁷⁾.

239 These studies however, used intravenous infusions of L-carnitine and insulin, with plasma carnitine
240 concentrations reaching supraphysiological levels. Thus, the logical progression was to investigate
241 whether oral carnitine supplementation can result in raised muscle carnitine content with dietary
242 carbohydrate used to stimulate insulin concentrations. The same group found that 3 g of L-carnitine
243 supplementation, with 4 boluses of 94 g sugar, resulted in reduced urinary carnitine excretion⁽⁶⁸⁾.
244 The authors hypothesised that, if the regimen were maintained for 100 d, then the result would be a
245 10% increase in muscle carnitine content.

246 Another group have performed a 14 d supplemental period with 3 g L-carnitine L-tartrate⁽⁶⁹⁾,
247 combined with a fairly high carbohydrate diet (approx 7 g/kg body mass/d). In contrast to the
248 proposition of Stephens *et al.*, lipid utilisation was not enhanced during cycling at 60% VO_{2max}. In
249 fact, carbohydrate oxidation increased by ~20% in male participants with no significant effects of
250 females (albeit showing a similar trend). A further study by the same group showed similar
251 results⁽⁷⁰⁾, with fat oxidation tending to be reduced after 2 weeks of L-carnitine L-tartrate
252 supplementation, compared to placebo.

253 An explanation for these discordant results may be provided by the exercise intensities employed.
254 In the first study to show that muscle carnitine content in humans can be elevated by carbohydrate-
255 carnitine co-supplementation, Wall *et al.* demonstrate that carnitine's influence on exercise
256 metabolism is dependent upon the intensity⁽⁷¹⁾. 24 weeks of supplementation with 2 g of L-carnitine
257 L-tartrate and 80 g of carbohydrate, consumed twice daily, increased muscle total carnitine content
258 by 21%. A control group consuming the same quantity of carbohydrate, with no carnitine
259 supplementation, showed no effect. A 55% reduction in muscle glycogen utilisation was found with
260 carnitine supplementation during cycling at 50% VO_{2max}. When the intensity was increased to 80%
261 VO_{2max}, no difference in muscle glycogen utilisation was observed, yet muscle lactate content was
262 44% lower, PCr/ATP ratio was better maintained. Pyruvate dehydrogenase activation status was
263 reduced during cycling at 50% VO_{2max} but increased at 80% VO_{2max} in the supplementation group,
264 versus control. A final, captivating observation was that, although body mass increased in the
265 control group (probably due to the 2512 kJ provided by the carbohydrate supplement; 600 kcal),

266 this was not evident in the carnitine group. Could the increase in fat oxidation have been apparent in
267 training and consequently account for the attenuation of weight gain? Determination of energy
268 intake would help to clarify this and could be an important aspect for future studies.

269 The implications of this for endurance performance are stirring. Previous attempts to spare muscle
270 glycogen via fat adaptation strategies have failed to enhance endurance performance, probably due
271 to a reduction in pyruvate dehydrogenase activation, thus impairing glycogenolysis when high rates
272 of carbohydrate flux needed to support intense exercise⁽⁷²⁾. Glycogen sparing would only be
273 successful if the spared stores are able to be “tapped into” towards the latter stages of a race. What
274 is the rationale of having more fuel if it is able to be used? This makes the findings of Wall *et al.* all
275 the more captivating, as carbohydrate utilisation is spared at low intensity exercise, but pyruvate
276 dehydrogenase activity was not downregulated at higher intensities, which would in theory enhance
277 performance. In practise, total work completed in 30 min increased by 11% after carnitine
278 supplementation, which confirms the idea that endurance performance can be enhanced. Further
279 studies must now investigate whether it is the glycogen sparing, acetyl group buffering, or any other
280 mechanisms at play which improve performance.

281 These findings shed new light on L-carnitine supplementation and opens up an exciting avenue for
282 future research. Notwithstanding this, as formerly mentioned, both the carbohydrate *per se*, and the
283 concomitant rise in insulin may counter the influence of the increased free carnitine pool, through
284 the increase in muscle glycogen and reduction in lipolysis. Therefore, although this practise may be
285 applicable to athletes who readily consume a high carbohydrate diet, it may not be advisable for
286 those with insulin resistance or diabetes to consume such a large amount of rapidly digestible
287 carbohydrates. A potentially suitable alternative could be by providing certain amino acids such as
288 glycine or leucine which are potent stimulators of insulin^(73; 47), thereby achieving the insulin-
289 stimulated uptake of l-carnitine with a lower carbohydrate load.

290

291 **Fish Oil**

292 Fish oils are rich in long-chain *n*-3 PUFAs. Supplementation with fish oil derived *n*-3 fatty acids
293 has been shown to reduce postprandial lipaemia⁽⁷⁴⁾. The increase in skeletal muscle lipoprotein
294 lipase activity⁽⁷⁵⁾ is probably the major mechanism behind this. Moreover, rodent models have also
295 demonstrated a protective effect of fish oil on high fat diet induced obesity, despite no differences
296 in energy intake⁽⁷⁶⁾. Taken together, these findings indicate that lipid utilisation is increased. In

297 addition, some have proposed that *n*-3 induced insulin sensitivity would enhance glycogen storage,
298 thus shifting the oxidation of carbohydrate onto lipids⁽⁷⁷⁾.

299 Major pathways in which *n*-3 fatty acids have been proposed to alter energy metabolism include;
300 providing a substrate for PPAR α ⁽⁷⁸⁾ and increases in skeletal muscle uncoupling protein 3⁽⁷⁹⁾.
301 Transcription of several genes involved in metabolism are also influenced by fish oil ingestion such
302 as carnitine palmitoyltransferase, fatty acid binding proteins, fatty acid transporter and fatty acyl-
303 CoA synthetase, and malonyl-CoA⁽⁸⁰⁾. Effect on genes can happen in minutes which would support
304 the acute supplementation of fish oil. Fish oil improves endothelial function, and reduces
305 inflammation⁽⁸¹⁾. Thus, another putative pathway for enhancing lipid utilisation could be via
306 increased adipose tissue blood flow which may be a limiting factor for fatty acid availability during
307 exercise⁽⁴⁾. Increases in lipid combustion in resting humans have been reported^(82; 83) coinciding with
308 a 40% reduction in the insulin response to an oral glucose load⁽⁸²⁾. Exercise studies show an
309 additive relationship between exercise and fish oil, with the combination of both showing the most
310 preferential changes in body composition and the greatest improvements in postprandial lipaemia^{(84;}
311 ⁸⁵⁾. Thus exercise may potentiate the effects of fish oil supplementation, thus elucidating changes in
312 metabolism.

313 A number studies have investigated the influence of fish oil on energy metabolism during exercise.
314 One has shown a clear increase in fat oxidation during exercise after 4 g/d of fish oil
315 supplementation for 3 weeks⁽⁸⁶⁾. In this protocol, participants were studied during running at 60%
316 VO_{2max}, under 6 conditions; 1) in the postabsorptive state, 2) following a high fat meal, 3) following
317 a high fat meal supplemented with fish oil, 4) 5) and 6) as the first 3 trials, but after chronic
318 supplementation. The energy contribution from fat, estimated by indirect calorimetry, increased by
319 ~10%, post-supplementation. Delarue *et al.* also found a tendency for enhanced fat oxidation (by
320 ~7%) and reduced metabolic glucose disappearance during cycling at 60% VO_{2max}⁽⁸⁷⁾. These results
321 prove promising, yet, controlled studies would provide more tangible evidence, as a number of
322 factors could influence fat usage over the 3-week periods used between these tests.

323 A crossover study found no significant difference in fat oxidation during cycling at 50% VO_{2max}⁽⁸⁸⁾.
324 The participants in this study were examined with and without 7.2 g/d of fish oil supplementation
325 for 2 weeks, with a 6 week washout period. This was followed up with a parallel study by the same
326 group, with a fish and olive oil supplemented diet compared to an isoenergetic control diet⁽⁸⁹⁾.
327 Exogenous fat oxidation was estimated using labelled isotopes and cycling exercise was, again,
328 performed at 50% VO_{2max}. Whole-body fat oxidation was potentiated to a greater extent (42% vs

329 4%) in the intervention group, but from a lower baseline. Thus post-intervention, both groups
330 showed similar rates of fat utilisation. Exogenous fat oxidation on the other hand, showed a trend to
331 be increased in the intervention group compared to the control group, post supplementation.

332 Fish oil supplementation has a plausible rationale for influencing substrate metabolism. A handful
333 of studies have shown trends and/or significant increases in lipid utilisation during exercise with
334 fish oil supplementation, yet evidence from randomised, crossover studies are still lacking to
335 provide the most concrete evidence. Until this clarity is achieved, it is unlikely that increasing fish
336 oil intake modestly (to around 4 g/d) is of detriment to athletes (recreational or elite) and may
337 provide some benefits to fat metabolism alongside other health outcomes. Therefore on a cost-
338 benefit decision fish oil could be taken as a dietary supplement.

339 **Calcium & Dairy**

340 The theoretical role of calcium and dairy products in modulating energy metabolism stemmed from
341 an anti-hypertensive study in obese African-Americans. An unexpected result was found, that
342 increasing calcium intake from 400 to 1000 mg/d through yoghurt consumption led to a reduction in
343 body fat of 4.9 kg over a 1 yr period. The link between calcium and fat mass has been confirmed by
344 a variety of both epidemiological and intervention studies (For review see ref.⁽⁹⁰⁾). Calcium intake
345 has also been associated with reduced risk of hypertension and insulin resistance. Of interest is that
346 dairy calcium appears to be 50-100% more effective for body fat loss than calcium alone⁽⁹¹⁾.

347 Although part of its effects are probably due to reduced absorption of dietary fat⁽⁹²⁾, a theory has
348 been devised explaining a potential link between calcium intake and substrate utilisation. Zemel⁽⁹³⁾
349 developed a hypothesis based on animal and cell models that an increase in calcium intake can
350 mediate intracellular calcium concentrations within adipocytes via reductions in 1,25-
351 dihydroxyvitamin D₃ (calcitriol) and parathyroid hormone (PTH) concentrations⁽⁹³⁾. Intracellular
352 calcium has long been associated with insulin resistance in obesity⁽⁹⁴⁾, but is also thought to be a
353 key regulator in adipocyte lipolysis and lipogenesis. Calcitriol may also act via other pathways,
354 including the nuclear Vitamin D receptor to downregulate uncoupling protein 2 thereby potentially
355 influencing thermogenesis and b-oxidation. Although the majority of this work has been conducted
356 on adipocytes, a high calcium diet has also been shown to increase uncoupling protein 3 expression
357 in the skeletal muscle of mice, also acting via calcitriol suppression. PTH can also inhibit CPT1
358 activity, a rate limiting step discussed in reference to L-carnitine supplementation. Moreover, recent
359 work has demonstrated calcitriol suppression of mitochondrial biogenesis and increased cytokine
360 production^(95; 96). Both of which are known to alter energy metabolism.

361 The manipulation of postprandial fat oxidation by calcium intake in humans, has been reviewed
362 elsewhere⁽⁹⁷⁾. Thus, only studies involving exercise will be discussed presently.

363 Both acute and chronic endurance exercise results in shifts in calciotropic hormone concentrations
364 (For review see Maimoun & Sultan⁽⁹⁸⁾). This is most likely due to sweat calcium losses. Therefore,
365 the metabolic effects of calcium intake may alter during exercise compared to rest and warrants
366 research in this area. Indeed, it is possible to attenuate the effect of exercise on calciotropic
367 hormones with calcium ingestion prior to exercise⁽⁹⁹⁾, yet substrate utilisation was not measured, as
368 bone turnover was the main outcome in this study.

369 The earliest human study to identify a link between calcium and fat oxidation showed an
370 association between acute calcium intake and subsequent fat oxidation⁽¹⁰⁰⁾. This was followed up by
371 an intervention study where subjects consumed low (500 mg/d) or high (1400 mg/d) dairy calcium,
372 energy-balanced diets⁽¹⁰¹⁾. These diets were matched for energy, macronutrients, fibre, and
373 saturated:monounsaturated:polyunsaturated fatty acid ratios. Subjects completed 6 days of each
374 diet, twice in a randomised, crossover design. On day 7 of each trial, a room calorimeter was used
375 to study energy expenditure and substrate utilisation under energy balance and energy deficit
376 conditions. The energy deficit was achieved by modest calorie restriction (419 kJ; 100 kcal) with a
377 greater contribution from exercise energy expenditure (~2093 kJ; ~500 kcal). 24 h fat oxidation was
378 28% greater when subjects consumed the high calcium diet when in energy deficit versus the low
379 calcium diet in energy deficit. However, under energy balance, no differences were seen between
380 diets. Calcitriol concentrations were suppressed to a slightly greater extent in energy deficit by the
381 high calcium diet.

382 The effects of acute ingestion of calcium were studied in a group of trained female runners⁽¹⁰²⁾. Four
383 hours after consumption of a standardised meal containing 3 g carbohydrate/kg body mass, the
384 participants consumed a test beverage with a high (500 mg) or low (80 mg) calcium content. Mean
385 carbohydrate content was 117 and 118 g for the low and high dairy calcium beverages, respectively.
386 One hour following consumption of the test drink subjects ran on a treadmill for 90 min at 70%
387 $\text{VO}_{2\text{max}}$. This was followed by a 5-min break and a subsequent 10 km time trial. Neither RER, nor
388 fat oxidation differed between trials. Yet, fat oxidation was in minus figures at rest. This would
389 suggest that the high carbohydrate content of the prior meals was inhibiting fat oxidation to such an
390 extent that it induced net, whole body de novo lipogenesis⁽¹⁰³⁾, thus explaining no effect of calcium.
391 This is supported by the relatively low levels of fat oxidation observed during the exercise bout
392 (~0.2 g/min). Treadmill based exercise at a similar intensity elicits a rate of fat oxidation of ~0.5

g/min in a large group of women⁽¹⁾. Furthermore, resting studies suggest the availability of NEFAs appears to diverge at around 3-4 h after consumption of high vs low calcium meals⁽¹⁰⁴⁾. As the test drink was consumed 1 h prior to exercise, this may have been too short a time period for the effects to be apparent. A further limitation may have been the fairly high exercise intensity as mentioned earlier in the section covering GI. It should be acknowledged that resting studies which have demonstrated an increase in fat oxidation have generally compared high calcium, high vitamin D meals with low calcium low vitamin D meals^(105; 104; 106). Whether calcium alone is capable of augmenting fat oxidation, or whether the presence of vitamin D is required needs elucidating.

The authors proposed that another reason for no effect on fat oxidation was due to the exercise-induced increase in PTH, which is probably due to dermal sweat calcium losses. However, recent evidence suggests calcium feeding just 20 min before exercise can attenuate the effect of exercise on PTH by ~30% relative to placebo⁽⁹⁹⁾.

There exists a substantial amount of theory supporting the role of calcium intake in both muscle and adipocyte fat metabolism. Acute effects may result from an increase in adipocyte lipolysis, raising plasma NEFA availability and reduced inhibition of carnitine and long-chain fatty acid esterification. Chronic intake may result in increased mitochondrial density, although all these effects are yet to be seen in humans during exercise, which itself produces shifts in calciotropic hormone concentrations. More research is needed before any recommendations can be made, to establish whether calcium can influence fat oxidation during exercise. Studies should determine whether this occurs under different nutritional states with varying carbohydrate availability, habitual calcium intakes and vitamin D status.

414

415 **Caffeine**

Former reviews have concluded that there is little evidence of caffeine influencing fat oxidation during exercise^(9; 107). This is surprising, given the vast metabolic changes that caffeine consumption can bring about. These include decreased insulin sensitivity, glucose tolerance, and muscle glucose uptake, and increases in catecholamines and NEFA availability^(108; 109).

Enhanced fat oxidation, with subsequent glycogen sparing was originally thought to be the major mechanism behind the ergogenic potential of pre-exercise caffeine ingestion. Although the results of these early studies have not been recently replicated. Graham *et al.* attempted to overcome the problems of small sample sizes which have been apparent in muscle biopsy studies involving

424 caffeine ingestion and exercise⁽¹¹⁰⁾. Data were pooled from a couple of studies which increased the
425 sample size to 37. The caffeine intake was between 5 and 9 mg/kg body mass; a dose which is
426 almost certainly ergogenic⁽¹¹¹⁾. They found a tendency for reduced glycogen utilisation, but this was
427 not significant ($P=0.22$). This may be due to the high intensities of exercise used (70-85% $\text{VO}_{2\text{max}}$),
428 combined with the time point of the sample being only 10 and 15 min after exercise initiation. The
429 lower the intensity and the more prolonged the duration of exercise, the greater the reliance on
430 NEFAs⁽⁸⁾, thus the effect of caffeine of fat oxidation via lipolysis may be blocked at high intensities
431 of exercise. Moreover, it would be expected that any differences would only be augmented as
432 exercise continued, thereby potentiating the chances of evidencing a significant change.

433 Jacobson *et al.*⁽¹¹²⁾ aimed to tackle this by providing a high fat (1.2g/kgBM) meal combined with an
434 IV heparin infusion (to increase NEFA availability). This was compared to the same meal and
435 infusion in addition to caffeine (6mg/kgBM) ingestion, vs a high carbohydrate (2.6g/kgBM) meal
436 and high carbohydrate meal with caffeine ingestion. All meals were consumed 60 min prior to a
437 bout of 120 min cycling at 63% of peak power output. Although caffeine consumed with the high
438 fat meal increased circulating NEFA concentrations compared to the high fat meal alone. This
439 increase was only significant in the period prior to exercise, after this, there was a tendency for
440 NEFA concentrations to remain elevated but fat oxidation was unaffected.

441 Findings from a more recent study are at slight discord with that of Jacobson *et al.*⁽¹¹²⁾. Ingestion of
442 caffeine (800 mg) reduced RER and tended to increase fat oxidation by ~10% ($P=0.069$) during 2 h
443 of cycling at 50% of maximum power output with carbohydrate ingestion (60g/kg/h)⁽¹¹³⁾. When
444 carbohydrate intake during exercise was prevented, there was no difference in RER when caffeine
445 or placebo was consumed. Furthermore, muscle glycogen use was similar between all trials. Hence,
446 the support for augmented fat oxidation is not robust. That this study was conducted after 2 days of
447 reduced energy intake (~5103 kJ/d; ~1219 kcal/d) combined with exercise (2 h at 50% of maximum
448 power output) is a strength, as the results can be applicable to those who are looking to achieve a
449 negative energy balance to reduce body mass through dietary restriction and exercise.

450 Although the many metabolic effects of caffeine such as insulin resistance and reduced glucose
451 tolerance have been demonstrated various times at rest, exercise may influence the outcome of
452 caffeine ingestion. A single bout of exercise has been shown to attenuate the insulin resistance
453 caused by caffeine ingestion⁽¹¹⁴⁾. In fact caffeine, co ingested with carbohydrate after exercise,
454 leads to the highest rates of glycogen resynthesis (~60 mmol/kg d/w/h) ever reported (excluding
455 infusion studies)⁽¹¹⁵⁾. If more carbohydrate is being stored as glycogen rather than being oxidised,

456 then presumably more lipid would be used to fuel metabolism during this period, yet the rapid
457 replenishment of muscle glycogen would lead to a shorter period of AMPK-activated fatty acid
458 oxidation⁽¹¹⁶⁾. Higher serum NEFA concentrations have been noted with caffeine ingestion
459 following exercise⁽¹⁰⁹⁾, providing support for potentially enhanced fat oxidation. Unfortunately,
460 measures of substrate metabolism have not been noted in the post-exercise recovery period to date.

461 The effect of caffeine on fat oxidation may only become apparent at lower exercise intensities than
462 those used in studies to date. As previously mentioned, increased NEFA availability may only be an
463 important regulator in substrate metabolism at moderate exercise intensities. Thus, the application
464 of energy metabolism for endurance performance may be limited, although, for certain training
465 sessions performed at a lower intensity or for the recreationally active population, caffeine may be
466 able to enhance fat oxidation. Looking to the future, it appears reasonable to investigate caffeine in
467 combination with the polyphenols that naturally occur in beverages such as green tea. Not only does
468 this increase the applicability for those who obtain caffeine from drinking green tea, but a recent
469 meta-analysis⁽¹¹⁷⁾ has discovered that the caffeine-catechin mixtures can significantly increase fat
470 oxidation by 16% relative to placebo, whereas caffeine alone elicits a non-significant increase of
471 12%. Hence, exercise studies using this combination are justified.

472 **Conclusion**

473 Recent developments have transformed some of the previous conceptions regarding nutritional
474 modulation of substrate metabolism during and after exercise. Lowering the glycaemic index of the
475 pre-exercise meal can increase fat oxidation during exercise, although information regarding the
476 long-term effects of a low glycaemic index diet in combination with exercise are lacking. L-
477 carnitine supplementation can affect metabolism in an exercise-intensity dependent fashion, but
478 only when combined with carbohydrate ingestion to enable uptake into skeletal muscle which may
479 not be suitable for some population who may benefit from increased muscle carnitine content. It is
480 currently not completely clear whether calcium can affect fat utilisation in isolation and future work
481 should aim to tease out potential integrative effects with vitamin D. Fish oil-derived n-3 fatty acids
482 have credible theory and some evidence of increased exogenous lipid oxidation during exercise yet
483 conclusive evidence of increase whole-body fat oxidation is not yet present. Similarly, caffeine has
484 been shown to stimulate NEFA availability and fat oxidation under certain dietary conditions, and
485 co-ingestion with catechins may modulate or potentiate the effectiveness of caffeine.

486

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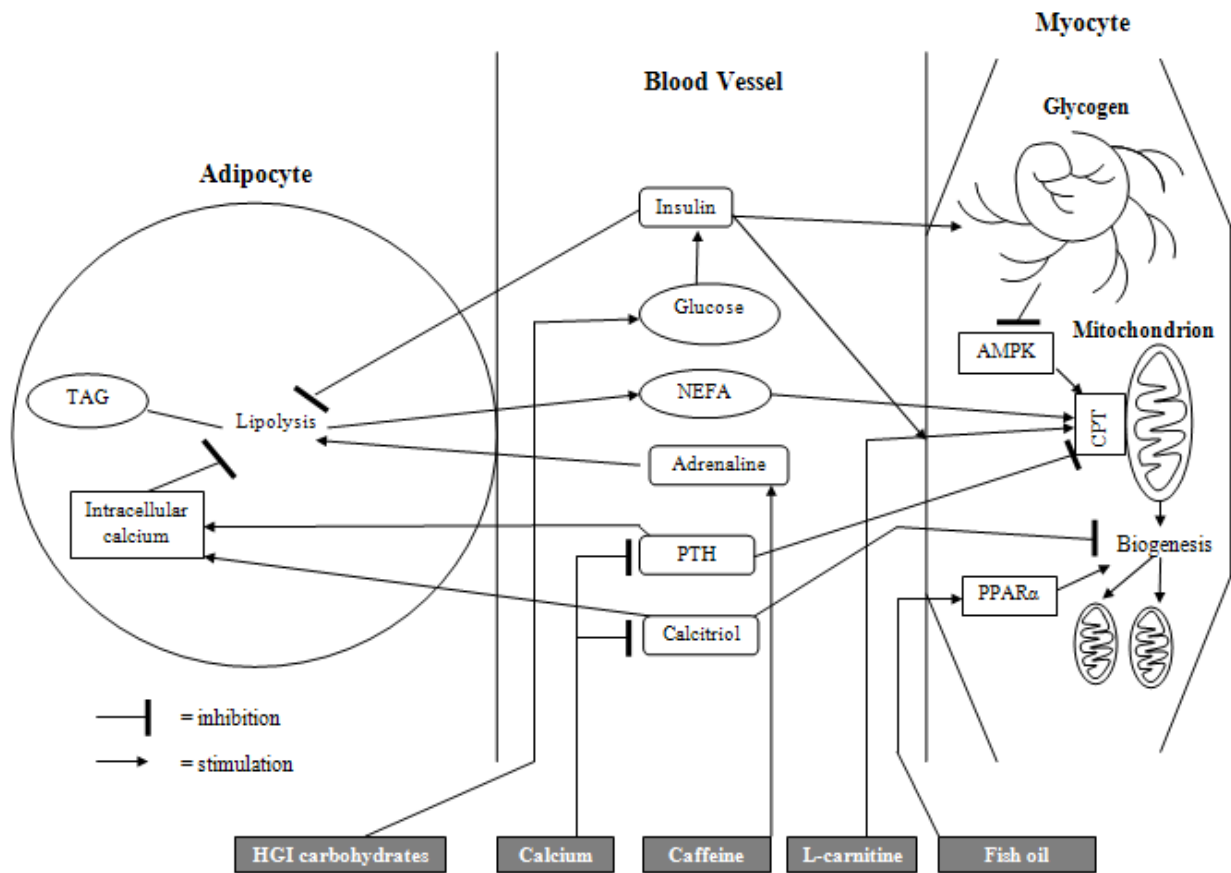
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773 caffeine on energy expenditure and fat oxidation: a meta-analysis. *Obes Rev* **12**, e573-581.
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778 **List of figure captions:**



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780 **Figure 1.** Mechanisms through which nutritional components may influence substrate selection
781 during exercise. AMPK, AMP-activated protein kinase; Calcitriol, 1,25-dihydroxyvitamin D₃; CPT,
782 carnitine palmitoyltransferase; PTH, parathyroid hormone.